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RESEARCH

Be cautious to adopt a second CAR T-cell infusion after failure of CD19/CD22 cocktail CAR T-cell therapy in relapsed/refractory B-NHL

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Abstract

Chimeric antigen receptor (CAR) T-cell infusion (CTI) therapy has emerged as a breakthrough therapy in relapsed/refractory B-cell non-Hodgkin's lymphoma (R/R B-NHL), but a substantial number of patients still suffer treatment failure. Data on disease history, subsequent salvage therapies, and outcomes of patients who face treatment failure after the first CTI (CTI1) have not been reported in detail or systematically studied. Here, a retrospective analysis was performed on a total of 61 R/R B-NHL patients in whom salvage therapies were adopted after CTI1 treatment failure, with their clinical characteristics, subsequent management and outcomes described in detail. The results suggested that second-time CTI (CTI2) used as salvage therapy after failure of CTI1 could achieve a better transient overall response rate (ORR) than other salvage treatments (non-CTI2) in only a minority of patients (8/27 vs. 2/34, P=0.014). Nevertheless, the non-CTI2 group showed better event-free survival (EFS) ($P = 0.007$) and overall survival (OS) ($P = 0.048$) than the CTI2 group, with a median follow-up of 6.7 months vs. 4.7 months. In addition, univariate and multivariate analyses showed that only the status of the tumor at disease onset was an independent risk factor for survival; salvage therapy after CTI1 treatment failure was not. The adverse effects of CTI2 treatment were generally similar to those of non-CTI2 treatment, but the infection-related mortality was considerably higher. In conclusion, the prognosis of patients who fail CTI1 therapy is very poor regardless of the subsequent salvage therapies, and clinicians should be cautious about adopting CTI2 treatment after failure of treatment with the CD19/22 cocktail CTI1 in R/R B-NHL. Large-scale prospective studies are warranted, and new strategies are urgently needed to prevent treatment failure and improve the survival of B-cell lymphoma patients in future.

Keywords CAR T-cell · B-NHL · Relapsed/Refractory · Salvage therapy · Adverse effects

Abbreviations

B-ALL	B-cell acute lymphoblastic leukemia;
B-NHL	B-cell non-Hodgkin's lymphoma
BR	bendamustine and rituximab
CAR	Chimeric antigen receptor
CR	complete remission
CTI	Chimeric antigen receptor T-cell infusion
CTI1	first chimeric antigen receptor T-cell infusion
CTI2	secondary chimeric antigen receptor T-cell infusion
ddPCR	droplet digital polymerase chain reaction
EFS	event-free survival
HSCT	hematopoietic stem cell transplantation
ORR	overall response
OS	overall survival
PFS	progression-free survival
PR	partial remission
PV	polatuzumab vedotin
R/R	relapsed or refractory

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Introduction

With the advent of the rituximab era, the prognosis of many B-cell non-Hodgkin's lymphoma (B-NHL) patients has improved substantially [1], but for relapsed or refractory (R/R) patients, prognosis is still poor, with long-term survival rates of only 20-40%, and effective treatment in many high-risk cases as well as for specific subtypes is lacking [2–4]. For patients with R/R B-NHL, the application of chimeric antigen receptor (CAR) T-cell immunotherapy, which has seen rapid advances in the field of hematologic oncology in recent years, has shown surprising efficacy. It has been shown that CAR T-cell infusion (CTI) therapy targeting CD19 can lead to remission or even recovery in most R/R hematologic patients [5, 6]. In addition to CTI therapy targeting CD19, CTI therapy targeting other common B-cell antigens, including CD22, CD20, and CD79b, has also emerged, achieving unexpected effects [7–9]. However, approximately 10-30% of patients do not respond to the first-time CTI (CTI1), and long-term follow-up has shown that 30-60% of patients also face relapse after adopting CTI1 [10–12].

To address the issue of treatment failure (failed to respond or eventually relapsed after a partial or complete response, PR/CR) after CTI1, researchers have proposed corresponding strategies, including optimizing CAR structure and improving costimulatory molecules to enhance the function of CAR T-cells [13–15], but most of these strategies are still at the preclinical stage, and their real efficacy has to be further validated in clinical applications. For these patients suffering treatment failure after CTI1, subsequent salvage therapy is already imminent and faces a great challenge. It has been shown that patients with B-NHL and chronic lymphocytic leukemia (CLL) who progressed after CTI1 therapy targeting CD19 had a very poor prognosis [12, 16, 17], and related retrospective studies suggested that second-time CTI (CTI2) as salvage therapy after progression could also only benefit a minority of patients [18–20]. In addition, small molecule-targeted drugs have also been applied as salvage therapy in several studies [21, 22]. However, there is still controversy regarding how to choose the subsequent salvage therapy for B-NHL patients who have failed CTI1 treatment. The difference in efficacy and adverse effects between different salvage therapies and prognosis resulting from different options has not been reported thus far, and therefore, there has been little realistic experience in optimizing salvage therapy for patients post-CTI1 treatment failure. With the development of CTI therapy, it is important to pay close attention to the special group of patients who have failed CTI1 treatment and explore potentially effective salvage treatments to improve their prognosis. Therefore, we enrolled 61 B-NHL patients who had adopted salvage therapies after failure of CD19/22 cocktail CTI1 as subjects for analysis, describing in detail their clinical characteristics, subsequent management and outcomes, comparing the efficacy and adverse effects between different salvage therapies, and analyzing the relevant risk factors affecting the outcomes and prognosis. Our was to explore better management strategies for patients post-CTI1 treatment failure and provide more guidance and reference for clinical practice.

Materials and methods

Patients

A retrospective analysis was performed on a total of 61 patients after failure of treatment with CD19/22 cocktail CTI1 (ChiCTR-OPN-16008526) between January 2017 and May 2022 hospitalized in the Department of Hematology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, China, with their characteristics and subsequent salvage treatment modalities described in detail [8]. This study was reviewed and approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, and registered in the China Clinical Trials Registry (ChiCTR-OPN-16008526). All enrolled patients provided written informed consent.

CAR T-cell production and study design

T-cells were obtained from patients by single harvest and then lentivirally transduced to express a third-generation specific CAR. CAR T-cells were expanded in vitro for approximately 14 days and then infused back into the patient successively over several days. Patients receiving CTI were pretreated with the FC regimen (fludarabine 25 mg/m2 and cyclophosphamide 20 mg/kg for 3 days) prior to infusion of CAR T-cells. Expression of CD19 and CD22 in the neoplastic B-cells of these patients was verified by immunohistochemistry or multiparameter flow cytometry (MFC). The presence of the corresponding targets of CAR T cells was confirmed before infusion.

Assessment of efficacy, toxicities, and event definition

Treatment response was assessed by CT or PET/CT scan at month 1, every 3 months from month 3 to month 24, and, based upon clinical need, after 24 months according to the International Working Group Response Criteria for Malignant Lymphoma [23]. Mutations were detected by

second-generation sequencing (NGS) using NEXTSEQ 550 (Illumina San Diego, CA, USA). Bulky diseases were defined as tumor masses ≥7.5 cm [24]. Physical examinations and clinical laboratory tests were performed regularly throughout the study, and adverse effects, including neurological events, were monitored continuously from enrollment [25]. CAR gene copy number was detected by droplet digital polymerase chain reaction (ddPCR) [26]. A poorer expansion of CAR T-cells meant that CAR gene copy could not be detected within 3 months after CAR T-cell infusion. CTI1 treatment failure referred to failure to respond or eventual relapse after PR/CR. Relapsed disease was defined as progression after achieving PR/CR on assessment of efficacy post-CTI1 treatment without the application of additional therapy. Refractory disease was defined as failure to achieve PR/CR on assessment more than 3 months after CTI1. Early relapse was defined as relapse at less than 3 months from the assessment of CR/PR post-CTI1 to relapse. In all time-to-event analyses, the time of salvage therapy onset after CIT1 failure was the starting point, and the analysis of overall survival (OS) for all patients was based on death or last follow-up, whereas the analysis of event-free survival (EFS) was based on disease recurrence or severe disease-treatment-related adverse effects or death, whichever occurred first. Progression-free survival (PFS) was analyzed with the earliest occurrence of disease progression or death as the endpoint, whichever occurred first. For patients without events, the cutoff time was the date of the last follow-up.

Statistical analyses

OS was calculated from the date of initiation of salvage therapy to the date of death or last follow-up. Survival analysis was estimated by the Kaplan–Meier method. Fisher's exact test was used for analysis of categorical variables, and the Wilcoxon rank sum test was used for analysis of continuous variables. Cox models were used for univariate and multivariate analyses of factors associated with survival, and factors with P values ≤0.10 in univariate analyses were included in the multivariate regression models. Two-tailed P values <0.05 were considered significant. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) 25.0 software (SPSS Inc., Chicago, USA).

Results

Baseline characteristics of patients

Between January 2017 and May 2022, 86 B-NHL patients suffered treatment failure post-CTI1, of which 61 patients were administered subsequent salvage therapies and were included in this study, with the other 25 patients adopting palliative care excluded (Fig. 1). Thirty-four of the 61 patients enrolled relapsed after CTI1 treatment, with a median relapse time of 2.2 months (range, 0.4-26.7), and the other 27 were resistant to CTI1 treatment; efficacy could be evaluated in all of these patients. Fourteen of the 34 relapsed patients received CTI2 as salvage therapy, while 13 of the 27 refractory patients received CTI2 as salvage therapy. The remaining patients were administered other salvage treatments (non-CTI2), including radiotherapy, conventional chemotherapy, and molecular targeted drugs. Detailed characteristics of patients treated with different salvage therapies are summarized in Table 1. No significant differences were found in the baseline characteristics, such as age and sex, between the two groups, and the median follow-up time was 4.7 months (range, 1.1-53.0) in the CTI2 group and 6.7 months (range, 3.0-45.5) in the non-CTI2 group.

Efficacy and outcomes

The efficacy of different salvage therapies after CTI1 treatment failure

To explore the effective salvage therapy after treatment failure of CTI1, the response rate was compared in the CTI2 and non-CTI2 groups at different time points. The results showed that the CTI2 group achieved a higher overall response rate (ORR) at the 30-day assessment (8/27 vs. 2/34, $P = 0.014$) than the non-CTI2 group, but no significant difference was found at other times (Fig. 2). In addition, a subgroup analysis was performed for relapsed and refractory patients adopting CTI2 as salvage therapy, and the results showed that there was no difference between these two groups (Supplementary Fig. S1).

The prognosis of patients with different salvage treatments after treatment failure of CTI1

To investigate the effect on prognosis for different salvage treatments after CTI1 treatment failure, statistical analyses for prognostic indicators in the two groups of patients were conducted. No significant differences were found in PFS between these two groups ($P = 0.057$), but patients in the non-CTI2 group showed a better EFS ($P = 0.007$) and OS ($P = 0.048$) than patients in the CTI2 group (Fig. 3).

Factors associated with the efficacy of different salvage treatments and prognosis in different groups of patients

To explore the relative factors affecting the efficacy of salvage therapies as well as the prognosis of patients, corresponding univariate and multivariate analyses were performed, showing that bulky disease ($P = 0.019$) and

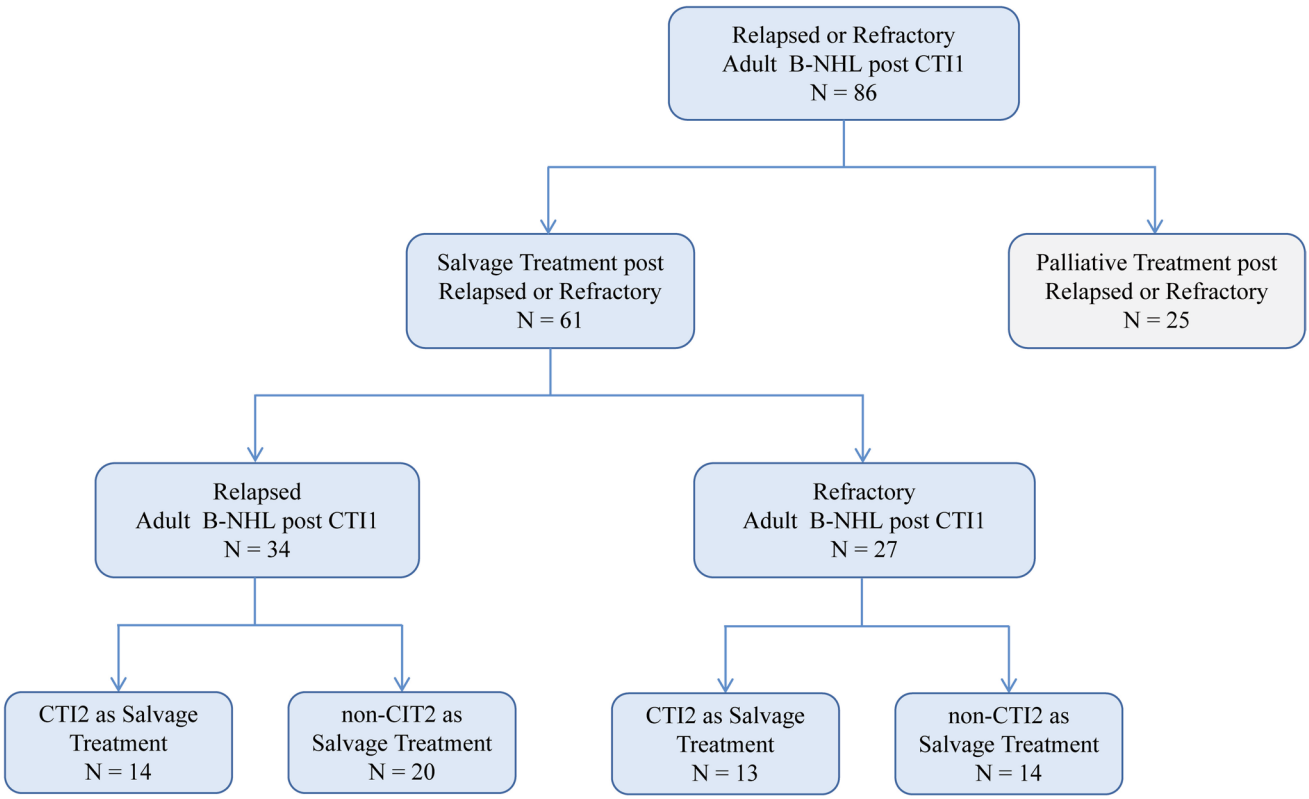

Fig. 1 Flowchart of enrolled patients

the best response to CTI1 ($P = 0.006$) were the factors related to the best ORR. Specifically, better response to CTI1 achieved and absence of bulky disease were probably associated with better outcomes of salvage therapy. However, the means of salvage therapy did not affect the best ORR of patients ($P = 0.279$) (Supplementary Fig. S2). Univariate analysis showed that Ann Arbor stage ($P = 0.017$), bulky disease ($P < 0.001$) and salvage therapy ($P = 0.007$) were risk factors for EFS, and multivariate analysis suggested that Ann Arbor stage ($P = 0.003$), bulky disease ($P < 0.001$), best response to CTI1 ($P = 0.019$) and salvage therapy ($P = 0.020$) were independent risk factors for EFS (Supplementary Fig. S3). The univariate analysis related to PFS found that bulky disease ($P < 0.001$) was the risk factor affecting PFS, and the multivariate analysis also found that only bulky disease ($P < 0.001$) was an independent risk factor (Supplementary Fig. S4). A univariate analysis for OS discovered that Ann Arbor stage ($P = 0.003$), bulky disease ($P = 0.001$), bridging HSCT with CTI1 ($P = 0.035$), best response to CTI1 ($P = 0.023$) and salvage therapy ($P = 0.048$) were risk factors, and the multivariate analysis showed that only Ann Arbor stage ($P = 0.029$) and bulky disease ($P = 0.008$) were independent risk factors for survival (Supplementary Fig. S5).

Relationship between CAR copy number and the efficacy of CTI2

In addition, the relationship between the efficacy of CTI2 salvage therapy and CAR copy number was investigated. The results showed that there was no significant difference in the peak copy number of CAR T-cells between patients who did or did not respond to CTI2 (Fig. 4A), but CAR T-cell expansion at 3 months was better in patients who responded to CTI2 than in those who did not respond ($P = 0.012$) (Fig. 4B).

Efficacy of different non-CTI2 salvage treatments and prognosis in patients

To explore the appropriate salvage therapy, the ORR (Fig. 5A) and OS (Fig. 5B) were compared in groups of patients treated with different non-CTI2 salvage treatments, and no statistically significant differences were found between these six groups.

Table 1 Baseline characteristics of patients treated with different salvage treatments post-CTI1 treatment failure

Characteristics	CTI2 as Salvage (N=27)	Non-CTI2 as Salvage (N=34)	*P* Value
Age, median (range), years	42 (25-65)	50 (17-72)	0.154
Gender			0.624
Male	15	21	
Female	12	13	
Underlying diseases			1.000
DLBCL	23	30	
MCL	2	2	
MZL	1	1	
FL	1	1	
At disease onset			
Ann arbor stage≥Ⅲ	24	33	0.313
IPI score ≥3	19	20	0.351
Bulky disease (≥7.5 cm)	7	5	0.274
Genetics			
TP53 disruption	12	8	0.084
Double hit	1	7	0.066
Bone marrow involved	10	6	0.087
Central nervous system involved	2	7	0.276
Lines of therapy prior to CTI1, median (range)	5 (3-8)	5 (3-9)	0.284
HSCT prior to CTI1	5	6	0.930
Bridging HSCT with CTI1	6	7	0.877
Best response of CTI1			0.794
CR	6	10	
PR	8	10	
NR	13	14	
Follow-up time, median (range), months	4.7 (1.1-53.0)	6.7 (3.0-45.5)	0.245

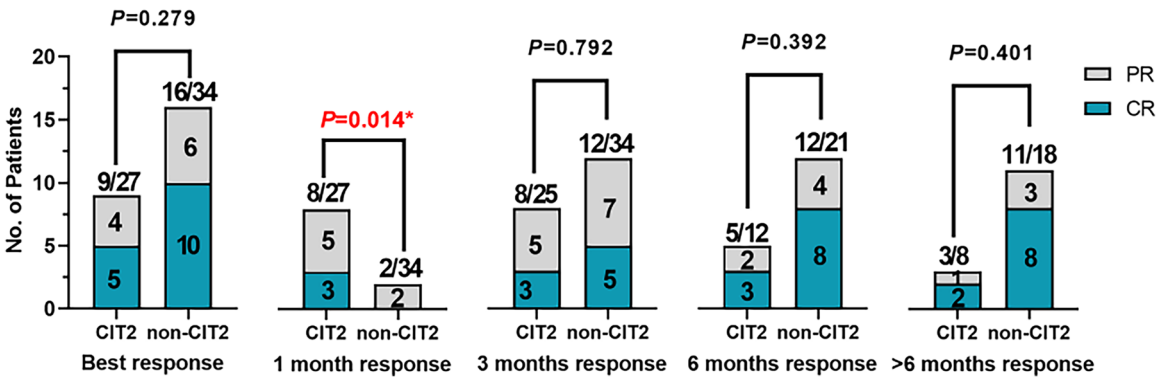

Fig. 2 Responses of patients treated with different salvage treatments after treatment failure of CTI1

Adverse effects

All patients were evaluated for adverse effects, including hematologic toxicity, hepatic and renal toxicity, cardiac toxicity, neurologic toxicity, and serious infections after salvage therapies. There were no statistically significant differences observed in the majority of common adverse effects between the CTI2 group and the non-CTI2 group. (Table 1).

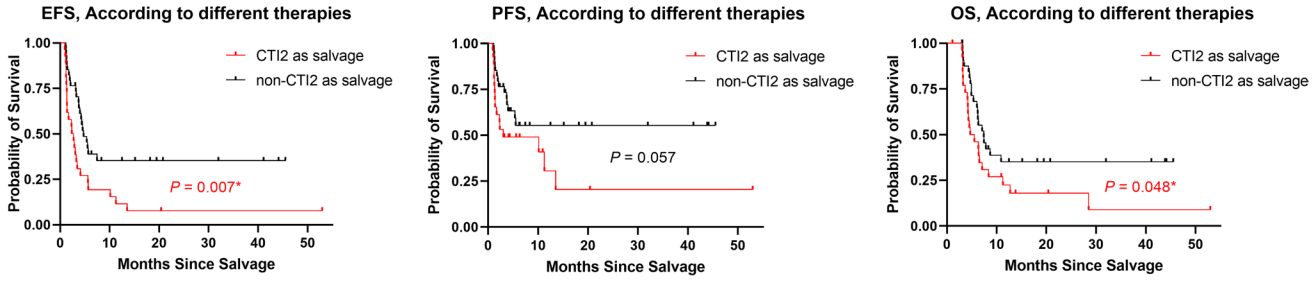

Fig. 3 Outcomes of patients treated with different salvage treatments after treatment failure of CTI1. **A** EFS of patients according to different salvage therapies; **B** PFS of patients according to different salvage therapies; **C** OS of patients according to different salvage therapies

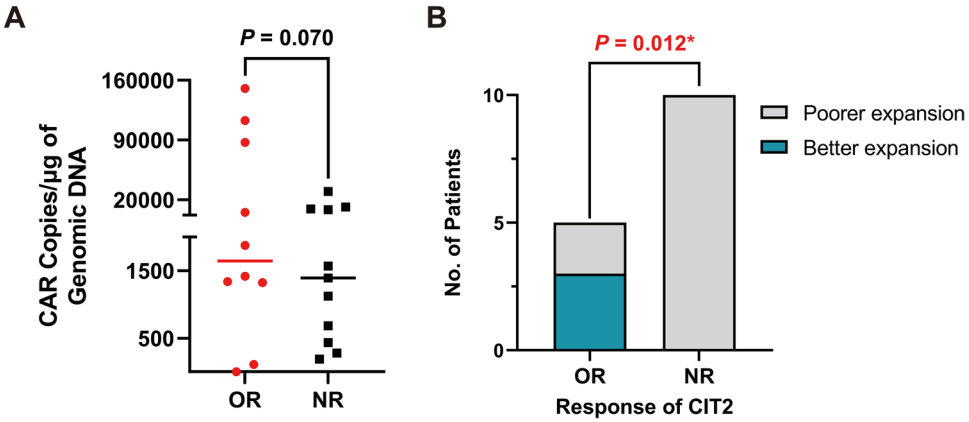

Fig. 4 Relationship between the efficacy of CTI2 as salvage therapy and the number of CAR copies. **A** relationship between the efficacy of CTI2 as salvage therapy and the peak CAR copy number; **B** relationship between the response of CTI2 as salvage therapy and the 3-month CAR T-cell expansion

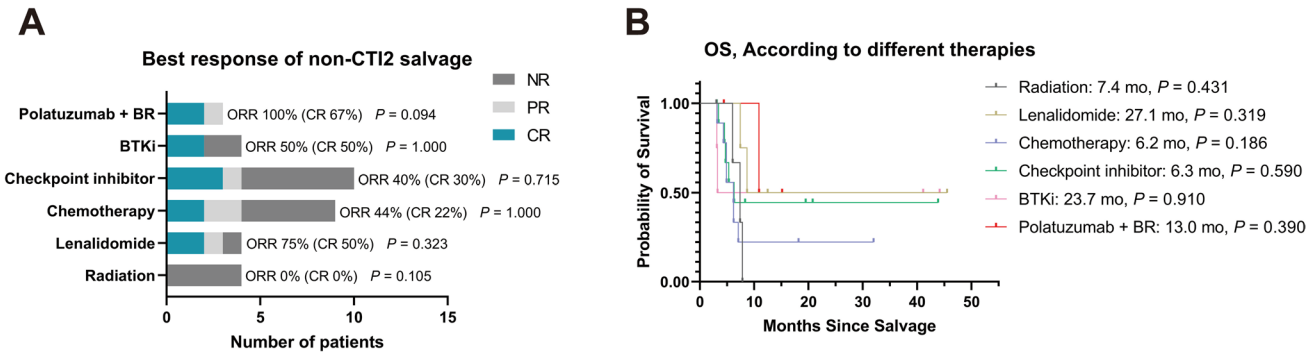

Fig. 5 Responses and outcomes of patients treated with different non-CTI2 salvage treatments after treatment failure of CTI1. **A** responses of different non-CTI2 salvage therapies; **B** outcomes of different non-CTI2 salvage therapies

However, the incidence of infections ≥ grade 3 was significantly higher in the CTI2 group compared to the non-CTI2 group (18/27 vs. 12/34, $P = 0.021$). Additionally, infection-related mortality was also elevated in the CTI2 group (13/22 vs. 4/19, $P = 0.025$). Bacterial infections were the most frequent, followed by viral and fungal infections, with no significant difference in the types of infections between the two groups. IgG levels were compared between the CTI2 group and the non-CTI2 group. Hypogammaglobulinemia (defined as IgG < 400 mg/dL) was observed in 4 patients (16%) in the non-CTI2 group and in 8 patients (42%) in the CTI2 group. No obvious difference regarding the IgG lowest value, the time to the lowest value and hypogammaglobulinemia was observed between the two groups, as detailed in Supplementary Table 1 and Supplementary Figure 6.

Discussion

Previous studies have shown that patients with R/R B-NHL who fail CTI1 therapy have an extremely poor prognosis [10, 12, 16, 17], but the potential benefits from salvage therapies,

Adverse effects of patients treated with different salvage treatments post-CTI1 treatment failure

Events	CTI2 as Salvage N=27	Non-CTI2 as Salvage N=34	*P* Value
Hematological toxicity			
Neutropenia (III/IV)	21	22	0.266
Anemia (III/IV)	15	17	0.666
Thrombocytopenia (III/IV)	14	14	0.406
Hepatotoxicity ≥grade 2	3	2	0.647
Nephrotoxicity ≥grade 2	1	0	0.443
Cardiotoxicity ≥grade 2	0	1	1.000
Cytokine release syndromes			0.920
CRS grade 0	6	8	
CRS grade 1	12	13	
CRS grade 2	6	7	
CRS grade ≥3	3	6	
Neurotoxicity ≥grade 2	1	1	1.000
Infections ≥grade 3	18	12	0.021*
Type of infection			0.389
Bacterial	9	10	
Viral	4	1	
Fungal	3	1	
Mixed infection	2	0	
Death associated with infections	13/22	4/19	0.025*

including radiotherapy, chemotherapy, hematopoietic stem cell transplantation (HSCT), and CTI2, still exist. No studies have yet demonstrated the better efficacy and long-term benefit of CTI2 as salvage therapy compared to other treatments, making the choice of optimal salvage therapy for this special group of patients an enormous challenge. Our study illustrated the extent to which different salvage therapies could benefit patients by analyzing the outcomes of different salvage treatments and highlighted the urgency for innovative approaches to improve the efficacy of CTI treatment and prevent relapse.

CTI2 as salvage therapy after treatment failure of CTI1 could only achieve a better transient ORR than non-CTI2 treatment in several patients, while the long-term outcomes did not differ from non-CTI2 treatment, with only the status of tumor at disease initiation being an independent risk factor affecting the prognosis of patients

Management strategies post-CTI1 treatment failure varied widely, with some patients adopting CTI2 or palliative radiotherapy combined with salvage chemotherapy or not, but ORRs for these patients were low regardless of the choice of salvage therapy, and even when CR or PR was achieved, remission was quite brief and could not be durable. The median OS of all patients suffering from CTI1 treatment failure was 6.3 months (95% CI: 2.5-39.2), indicating that the prognosis of these patients was extremely poor, consistent with previous reports.

By comparing different salvage treatment modalities between groups, we found that the CTI2 group achieved a higher ORR at the 30-day assessment (8/27 vs. 2/34, P=0.014), but subsequent follow-up showed no significant difference in ORR between the two groups, suggesting that the efficacy of CTI2 treatment might be more rapid but not better than that of non-CTI2 treatment. In addition, to assess whether CTI2 treatment could benefit a specific group of patients, a subgroup analysis was performed in refractory as well as relapsed patients and showed that the ORR achieved by CTI2 treatment was not different between the two groups of patients, either in patients who relapsed after CTI1 treatment or in patients resistant to CTI1 treatment, indicating that patients who failed CTI1 treatment could hardly benefit from CTI2. Recent studies have found that Th2 deficiency and overdifferentiation of the effector phenotype were significantly associated with CD19-positive relapse by single-cell multiomics analysis of CAR T products [27], suggesting that the immune microenvironment was also involved in relapse after CTI1 treatment, and there could be efficacy heterogeneity for CTI2 treatment in patients with such an immune microenvironment; therefore, small molecule-targeted drugs as salvage therapy rather than CTI2 treatment were more likely to benefit this group of patients.

Notably, survival-related analyses showed significant differences in EFS and OS among patients with different salvage treatment options, and the non-CTI2 group showed a better prognosis than the CTI2 group, suggesting that CTI2 not only failed to improve the prognosis of patients but also had a greater possibility of incidence of adverse events, leading to worse outcomes.

In our study, tumor size at disease onset appeared to be an important factor influencing the response to salvage therapy, as patients without bulky disease were more likely to respond. Additionally, bulky disease was identified as an important independent risk factor for EFS, PFS, and OS in survival-related analyses, suggesting that patients with bulky disease tend to have poorer outcomes. As the only independent risk factor for PFS, bulky disease suggested that patients so afflicted were more likely to experience disease progression, which was also consistent with the results of previous studies [28, 29], emphasizing the importance of tumor burden at disease onset for proper risk stratification and efficacy prediction. Best response to CTI1 was also a significant factor affecting the efficacy of salvage therapy, and the better response achieved with CTI1 treatment meant a

better response to salvage therapy, indicating that the tumor malignancy of patients resistant to CTI1 could not be ignored and that their responses to salvage therapy would be much poorer. In addition, the univariate analysis showed that Ann Arbor stage, best response to CTI1 and salvage therapy were also risk factors affecting EFS, and the multivariate analysis excluding confounding factors revealed that Ann Arbor stage, bulky disease, best response to CTI1 and salvage therapy were independent risk factors, indicating that patients with heavier tumor burden, poorer response to CTI1 and adopting CTI2 were more prone to events including disease progression and serious infections. The former three factors related to disease status were not difficult to understand in relation to prognosis, while CTI2 treatment as a risk factor implied that patients who adopted CTI2 as salvage therapy were more likely to experience serious adverse events. Notably, whether patients underwent HSCT prior to CTI1 treatment did not affect the response to salvage therapy. Although HSCT bridging CTI1 treatment was an influential factor for OS, it was not an independent risk factor in the multivariate analysis, suggesting that neither HSCT alone nor bridging CTI1 treatment could affect the prognosis of patients and that even if HSCT failed, some patients could still benefit from salvage therapy. Notably, related studies have found that polatuzumab vedotin (PV) showed a surprising effect as salvage therapy in patients who failed CTI1 treatment [22], and the PV plus bendamustine and rituximab (PV+BR) regimen was also applied as salvage therapy after CTI1 treatment failure in 3 patients in our study, 1 of whom was a refractory patient and the other 2 of whom relapsed after CTI1 treatment. Up to the end of this study, 2 of these patients achieved CR (67%, 2/3), and 1 patient achieved PR (33%, 1/3), suggesting that the PV+BR regimen might play an unexpected role as salvage therapy for patients who fail CTI1 treatment. Although statistically significant results could not be obtained due to the limitation of the sample size, further studies with more samples could help to draw a more accurate conclusion. However, the potential of molecular targeted drugs as salvage therapy for patients who have failed CTI1 treatment cannot be denied and might indicate an advantage over CTI2 treatment.

Many factors have been associated with the failure of CTI treatment, and corresponding strategies are being explored. In our study, it was revealed that the efficacy of CTI2 salvage therapy was not correlated with peak CAR T-cell copy number but with 3-month CAR T-cell copy number, although a large amount of follow-up data is needed to verify the current conclusion. However, this finding also indicated to some extent that the function of T cells and their continued proliferation in vivo were important factors influencing the efficacy of CTI treatment, consistent with the literature [30, 31], suggesting the importance of donor T-cell function testing prior to CTI to select a suitable donor and the need to confirm the expansion of CAR T-cells in vitro and functional testing prior to transfusion back to patients.

The adverse effects of CTI2 as salvage therapy were generally similar to those of non-CTI2 treatment, but the infection-related mortality was significantly higher

In addition to efficacy, the safety of different salvage therapies was also evaluated. Most patients experienced common antitumor adverse effects, such as myelosuppression, but serious adverse effects did not occur frequently, and there were no treatment-related deaths. No significant differences in most common adverse events, including CRS and ICANS, were observed between the groups.

However, the incidence of severe infections was greater in the CTI2 group compared to the non-CTI2 group, and the infection-related mortality was significantly higher than that in patients treated with non-CTI2 therapy. It was suggested that the immunosuppressive effect of CTI2 treatment may be generally stronger than that of non-CTI2 treatment, leading to weakened immunity and thus severe infection-related mortality, whereas patients adopting non-CTI2 developed severe infections but eventually recovered with supportive antibiotic therapy and autoimmune effects to avoid the outcome of death. B-cell aplasia and hypogammaglobulinemia are common immunological toxicities following CD19 CAR-T therapy, leading to immunocompromise [32]. No differences in IgG levels or hypogammaglobulinemia were observed between the CTI2 group and non-CTI2 group, indicating that the CAR T-cells effectively depleted normal CD19-positive B cells and suppressed IgG secretion. The elevated infection rate in the CTI2 group compared to the non-CTI2 group is likely attributable to the extensive immunosuppressive therapy, weakening both humoral immunity and cellular immunity. This suggests that the predisposing factors for infection following CAR T-cell therapy are multifactorial.

Our findings affirmed the poor prognosis of patients who failed CTI1 therapy and the importance and urgency of effective subsequent management. Our study also demonstrated that CTI2 as salvage therapy in patients who failed CTI1 treatment showed no significant advantage over non-CTI2 treatment and could not provide long-term benefit to patients but increased the risk of adverse events and even death related to infections. In contrast, non-CTI2 salvage therapy could achieve better EFS and OS than CTI2 salvage therapy, although multivariate analyses showed that only factors related to the tumor itself at disease initiation were independent risk factors for prognosis and survival. Therefore, based on the results of this study, it can be concluded

that CTI2 treatment might not be the best option as salvage therapy for B-NHL patients who suffer from post-CTI1 treatment failure, considering the complexity and cost of CTI therapy, and clinicians should be cautious to adopt CTI2 as salvage therapy after failure of treatment with the CD19/22 cocktail CTI1 in R/R B-NHL. Notably, the choice of PV+BR regimen probably has an advantage in salvage strategies. In addition, future large-scale prospective studies are warranted, and we will continue to explore new approaches.

The uniqueness and innovation of this study

Our study has several unique strengths. This is the first and largest domestic study of management strategies for B-NHL patients who fail CTI1 treatment, providing detailed data on adult B-NHL patients with failure of CTI1 treatment, including the choice of subsequent salvage treatments, efficacy, and long-term follow-up. Our research compared CTI2 with non-CTI2 as salvage therapy, demonstrating that CTI2 treatment cannot provide long-term benefit to patients and that caution should be taken when adopting CTI2 as salvage therapy. In addition, the subgroup analysis of different non-CTI2 salvage treatments found a surprising efficacy of the PV+BR regimen, which could also provide some clues for the clinic despite the lack of statistically significant conclusions due to the limitation of sample size. Finally, our study focused on adult B-NHL patients, and the results related to B-ALL and pediatric patients might be different.

In conclusion, our study identifies the factors influencing the effectiveness of salvage therapy in adult patients with B-NHL after failure of CTI1 treatment. From a comparison of the outcomes of patients using different salvage therapies, it is suggested that patients suffering CTI1 treatment failure have very poor prognosis and should be cautious about adopting CTI2 as salvage therapy, which is costly and of little benefit and even has a high risk of fatal infection. Future research should be actively focused on seeking new therapeutic approaches to reduce treatment failure of CTI and improve survival of B-NHL patients, including the application of other immune cell therapies or bispecific monoclonal antibodies, exploration of new CAR targets and novel CAR constructs with high efficiency and low toxicity.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

Consent for publication All authors consent to the publication of this study.

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